

Applications of Machine Learning Methods to Quantifying Phenotypic Traits that Distinguish the Wild Type from the Mutant *Arabidopsis Thaliana* Seedlings during Root Gravitropism

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Abstract—Post-genomic research deals with challenging problems in screening genomes of organisms for particular functions or potential for being the targets of genetic engineering for desirable biological features. ‘Phenotyping’ of wild type and mutants is a time-consuming and costly effort by many individuals. This article is a preliminary progress report in research on large-scale automation of phenotyping steps (imaging, informatics and data analysis) needed to study plant gene-proteins networks that influence growth and development of plants. Our results undermine the significance of phenotypic traits that are implicit in patterns of dynamics in plant root response to sudden changes of its environmental conditions, such as sudden re-orientation of the root tip against the gravity vector. Including dynamic features besides the common morphological ones has paid off in design of robust and accurate machine learning methods to automate a typical phenotyping scenario, i.e. to distinguish the wild type from the mutants.

Keywords: gravitropism, machine learning, phenotypic traits

1. Introduction

Systems biology has rapidly advanced to offer a number of powerful methods for discovery of networks of genes/proteins and collective functions of families of genes/

proteins in life processes. Analysis of Quantitative Traits Loci (QTL) refers to a systematic method for discovery of genes and their functions in systems biology. The major bottleneck in QTL progress appears to be in the more general and challenging question of how to quantify phenotypic traits, such as morphology and dynamics of its variation. QTL and other gene function discovery methods rely on quantification of phenotypic traits that could be observed and used in a systematic way to distinguish between organisms with differing DNA sequences or epigenetic signatures. To extract phenotypic traits that distinguish genotypic characteristics, biologists make repeated observations of the wild type and the mutants of the same species during growth, behavior or in the course of response to external stimuli. In the following, we illustrate and outline the steps for identifying and quantifying phenotypic traits of seedling of the model plant *Arabidopsis Thaliana* subject to changes in gravitational force relative to its natural root orientation during a normal course of growth. It will be demonstrated that the selected quantitative traits are together sufficiently informative to carry genomic signatures that are at work differently in the wild type and mutant plants.

2. Background and Biological Significance

Plant roots vary a great deal in morphology, size and complexity of their architecture. The model plant *Arabidopsis Thaliana* has a root system that is exemplary in both prevalence of its morphological pattern and its relative simplicity. There are several visible morphological features of this root system. The primary root is the main branch that starts its life immediately after germination. Besides being a significant method for simplifying morphological measurement, midline extraction serves as an example of high-dimensional data transformation that achieves dimensionality reduction with some loss of information beyond noise, namely, ignoring the signal-parameters underlying phenotypic variation such

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as morphology of the root due to image texture or image structures such as shadows of root hair. In higher resolution images, the interpretation will change, and root hairs could also be part of the features whose morphological diversity and variation in growth dynamics become essential biological quantities to be studied that are related to specific gene functions, pathways or gene-protein network dynamics. In this article we provide details of a set of algorithms for extraction of root and hair growth information.

Tropism refers to the directed growth responses of plants to external stimuli such as gravity, water, light, and temperature. Beginning with the pioneering work of Darwin, the study of tropism has grown to a major area of research in plant biology in the course of its century-long history. Darwin observed that grass seedlings grown in dark tend toward a light source when illuminated from one side. Plant roots show a similar response to gravity. When a seed germinates, the roots penetrate the soil and grow downward. However, if a root is reoriented by 90° with respect to the gravitational field, the root responds by altering its direction of growth, curving until it is again vertical. Sachs was the first one to propose a quantitative measure for gravitropism [32], namely, the gravitropic response was proportional to the component of the gravity vector perpendicular to the root axis. Early studies, using maize roots, demonstrated distinct regions along the root axis with different physiological response patterns. Despite this long and illustrious research history, the molecular mechanisms involved in sensing the gravitational signal and its transduction still need to be studied. Other tropic responses are sources of gaining information about the physiological and molecular processes that influence the tropic response, e.g. the plant hormone auxin has been implicated in tropic responses through extensive research by a broad group of scientists ([33], [6], [26]).

Auxin is involved in asymmetric tropic growth, vascular development and root formation, as well as in a plethora of other processes. The approaches by which auxin has been implicated in tropisms include isolation of mutants altered in auxin transport or response with altered gravitropic or phototropic response, identification of auxin gradients with radiolabeled auxin and auxin-inducible gene reporter systems, and by use of inhibitors of auxin transport that block gravitropism and phototropism. Proteins that transport auxin have been identified and the mechanisms which determine auxin transport polarity have been explored. The mechanisms of auxin action in the gravitropic response and phototropism have recently been revealed by the analysis of mutants that are defective in the response to the gravity signal, or have a characteristic response to blue and other spectral bands of light. Gravitational stimulus induces curvature of the primary root of *Arabidopsis Thaliana*. It is known that the patterns of root curvature reveal quantitative traits that are associated to a number of genes and proteins that play vital roles in growth and development of the plant.

3. Instrumentation and Methods

The application described below gives an outline of machine-learning methods that distinguish mutant from wild type seedling plants in gravitropism experiments. We note that these applications could be adapted for other experimental protocols in plant biology that attempt to quantify subtle phenotypic traits in order to decipher functions of genes-proteins.

3.1 Automation and High Throughput Imaging

We have designed and engineered a complete system, specifically focusing on high throughput plant imaging. This hardware-software product is a novel “*Portable Modular System for Automated Image Acquisition*” in the lab and for certain field experiments. Our model system is described in [4]. The algorithms below are part of “The Image Analyzer” component of our system. It is an object-oriented software application that can be modified to accommodate essentially any reasonable analysis scenario. The Image Analyzer package is developed in Linux, and it is ready for experimental protocols by the biologist, see [11]. This platform can provide high throughput results in a range of resolutions, and accommodates highly flexible experimental protocols. This article demonstrates the feasibility of using our system towards fully automated high throughput phenotyping in functional genomics and systems biology (Figure 1).

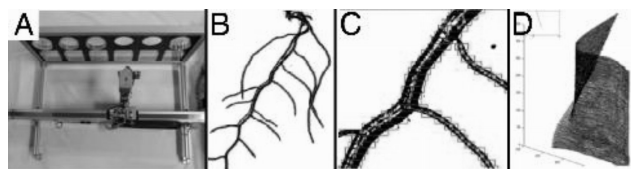


Fig. 1: [A] Top view of the experimental fully automated imaging device engineered in collaboration with PBC Linear Co. [B,C] Parts of a high resolution image of growth of lateral roots and extraction of morphological features through the algorithms developed by the authors. [D] The midlines of primary root images frames (please see below) could be metaphorically positioned on top of each other to visualize the dynamics of growth in gravitropism as the geometry of the surface. We have developed algorithms for extraction of midlines of root hair and dynamics of growth as the next step in capturing phenotypic variation.

3.2 Automated Preprocessing

In earlier contributions by the senior author and Liya Wang ([43], [44]) image analysis and midline extraction were only partially automated and required “*preprocessing tasks*” prior to application of the software. Preprocessing steps are highly data-dependent image processing steps. In this article, we report successful automation of the preprocessing, and a novel collection of algorithms that are also amenable to generalization beyond the plant roots, as well as parallelization. The preprocessing methods are adapted from earlier seminal research by Osher et al ([31], [17],

called the Total Variation regularization (TV) method. We remark that comparable approaches have been also treated in literature using Bounded Variation (BV). As expected, direct application of one set of programs to another typically shows varying degrees of success. Therefore, it is anticipated that generalization of the automation preprocessing would depend on the setting for image acquisition and would vary in degrees of successful analysis in plant growth dynamics of various kinds. The hardware and automation offered by CyPlant Solutions Inc. have a standardized setting that removes a series of technical issues in order to ensure the quality of data for the same genre of mathematical treatment as in our algorithms and software. Our work in progress will provide a critical examination of a number of algorithms and methods in literature, and in appropriate cases, redesigns the needed image preprocessing algorithms from scratch to be optimal for the tasks, such as those in CyPlant Solutions standardized technologies.

We next present the outline of the algorithm. Let the image for one gray-scale frame be represented by

$$f : J \rightarrow \mathbb{R}.$$

The basic idea is as follows: the typical image $f : J \rightarrow \mathbb{R}$ is written as an outcome of an unknown convolution kernel K with compact support (e.g. a discrete Gaussian), applied to the original image φ (or a desirable model that in our case must be suitable for segmentation and midline recovery of the branched structures, cf. [44]), and an additive noise ν , that we propose to model as a Gaussian white noise, such that

$$f = K * \varphi + \nu.$$

The automation algorithm selects the kernel and a suitable model for noise (that we must confront in practical applications), which will be derived from the High Throughput Imaging System outputs in lieu of the simplifying assumption above. We will begin with the constrained minimization problem with appropriate norms, without further mention of simplicity.

For a class of images obtained by our method, the higher resolution allowed us to select a delta function, and use the simpler form. However, in the case of animal behavior, blurring occurs due to movement of the animal or temporal loss of the automated focus. In the case of plants, blurring occurs due to accumulation of moisture on inside surfaces of the Petri dish covers. This means that we must use the general form below:

$$\varphi = \operatorname{argmin}_{\phi} \left\{ F(\phi) := \frac{1}{2} \|K * \phi - f\|_{L^2}^2 + c_1 \|\phi\|_{BV} \right\}.$$

Here, c_1 is a scaling constant that is the trade-off between noise and the desired image quality, such as having sharp edges in images of root systems, and arg-min is over all images ϕ , where φ is the desired form of the image suitable

for algorithms of [44], [30], [41], [9] (for an iterative regularization to recover finer scales). An intermediate problem that we solved numerically was the parallel implementation of methods based on the following idea that is adapted from a simplified mathematical result by Chan-Wong-Kaveh-Osher et al. that amounts to a constrained optimization problem with F as a function of joint variables (ϕ, K) :

$$(\varphi, \kappa) = \operatorname{argmin}_{\phi, K} \left\{ F(\phi, K) := \frac{1}{2} \|K * \phi - f\|_{L^2}^2 + c_1 \|\phi\|_{BV} + c_2 \|K\|_{BV} \right\}.$$

When we view F as a one-variable functional depending only on K or only ϕ , by fixing the other one, then F is convex, while $F(\phi, K)$ fails to be jointly convex. To overcome this difficulty, we followed [30] and instead numerically solved the two Euler-Lagrange equations, where the L^2 -conjugates are denoted by the “hat”:

$$F_{\phi}(\phi, K) = \hat{K} * (K * \phi - f) - c_1 \nabla \frac{\nabla \phi}{|\nabla \phi|} = 0,$$

$$F_K(\phi, K) = \hat{\phi} * (\phi * K - f) - c_2 \nabla \frac{\nabla \phi}{|\nabla \phi|} = 0.$$

Fixing K first, we solve for ϕ from the first equation and then switch the roles of K and ϕ and exchange the two equations. For a class of images according to our protocol, the higher resolution allows one to select an iterative scheme that mimics convergence to the appropriate delta function, which in turn allows one to use the simpler form mentioned above. However, in the case of movement of the entire root by sliding on the agar surface, blurring sometimes occurs; this could also be a result of temporal loss of the automated focus. As mentioned before, in the case of plants, blurring mostly occurs due to accumulation of moisture on inside surfaces of the Petri dish covers. In our companion article [37], and in our 2008 WorldComp article [43], and subsequent developments [17], [18], [38], we have developed a set of new algorithms and their object-oriented C-code for massively parallel-distributed hardware. This part of research was done in collaboration with nVIDIA and SUN Network.com.

3.3 Classification through Machine Learning

Having quantified morphological features from the data set of movies, we continue to discover candidates to serve as phenotypic traits that carry the plant genotypic signature that account for distinguishing between the wild type and the mutants. A number of these features depend on computation of the midline of root images as argued in [43]. The algorithm for midline tracing in the present work is different from the main algorithm in [43]. In fact, the second generation of midline tracing algorithms used in the present article is more robust, more accurate and much faster [37]. We computed midlines from different regions of a root image as follows.

Gravitropism results in anisotropic expansion of cell walls and an uneven change in the distribution of cells in the epidermis of the root in such a way that the root growth tends to generate the observed bending with respect to its growth in the original vertical direction [8]. The part of root before the bending region is referred to as the *horizontal region*. The bending region itself is called the *hook region* and finally, the part of the root after the hook region is called the *vertical region*. Lengths of these regions are calculated using the midline in these regions, and it serves as a feature. Besides the length associated to the above-mentioned regions, the curve made by the midline carries the information about the root curvature during its gravitropic growth. Further considerations (omitted here) indicate that the number of segments account for the changes in the growth direction which could potentially be a significant representative feature. The greatest change in the growth direction in the hook region is called the *hook angle*. In addition to the geometric features of the midline of the primary root growth, we also developed algorithms to extract growth information regarding the root hairs. Discussion of the biological significance and the algorithms for root hair growth information are available in [14], [13]. Let us only mention that the number of root hairs for each region of the root is also a feature that we added to the list of morphological features for the classification purposes. Using the root hair information, we formulate additional morphological features which we call *dynamic features* because they correspond to the growth velocity and growth acceleration of the primary root and the root hairs. Figure 2 shows these extracted features.

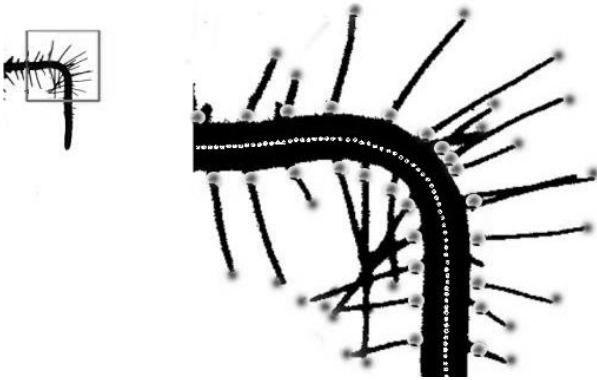


Fig. 2: Midpoints of a sample hook region. To avoid ambiguity of the image in hair areas, we just indicate the start and the end points of the hairs. For the hook region the midpoints are also shown.

All of the following features highlighted in the figure are considered as representative features of the growth images:

- length of the vertical region,
- length of the horizontal region,
- length of the hook region,
- the number of segments,

- the hook angle,
- the average root hair length,
- the number of root hairs,
- the root growth velocity,
- root growth acceleration,
- hair growth velocity,
- hair growth acceleration, and
- hair density.

From a human observer's standpoint, the slow rate of growth of the root morphological features results in "invisible phenotypic traits" that belong to the complex dynamical system underlying the development and growth. In [13] we have developed the preliminary steps towards the in-depth study of plant development within the framework of complex dynamical systems. For comprehensive details of these algorithms readers are referred to visit our website for [37], [13], [16], [12], [5] and [15].

To recap, we have used image analysis algorithms and mathematical modeling of dynamics of morphological features in order to isolate a number of quantitative morphological and dynamical features, and using these features, every movie from the growth process is assigned a representation by the appropriate vector, as described earlier. The final step towards discovery of quantitative phenotypic traits requires machine learning for classifying the above-mentioned features, and to train the machine through a set of so-called 'training samples' or 'examples' in order to extract the appropriate weighted combination of features that could carry genotypic signatures of the mutation (here, *mdr1*, which is one of the members of the *mdr* gene family, is knocked to provide the mutant). The machine learning method of choice here comes from the branch of statistical learning theory, called support vector machine (SVM), see [40], [39]. The flexibility of the SVM theory has proved advantageous here, because we tailored to our needs an RBF-based SVM, where RBF refers to the "Radial Basis Functions" as our choice for the SVM kernel function. We applied the RBF-SVM method to 281 seedling growth movies (161 mutant *mdr1* and 120 wild type seedlings) where 12 features captured them as described before. We used the MATLAB-SVM classifier [21] and calculated the precision of our classification outcome. Table 1 shows the results for each class of movies.

	Magnitude of random selected training data set	Precision
Class 0 (Mutant)	60	98.8
Class 1 (Wild Type)	45	86.4

Table 1: This table shows the result of applying the SVM method on the data set, where the radial basis function was used as the kernel function. Precision of the results shows efficiency of using the defined features for capturing different classes of genotypic attitudes.

The precision was computed via the standard error formula

$$\frac{\text{true positive}}{\text{true positive} + \text{false positive}}.$$

Since twelve features were employed we could not show a full 12-dimensional figure of the classes. Instead, the projection of the movies on the two coordinates corresponding to the features “Number of segments” and “Average hair growth acceleration” is shown in Figure 3.

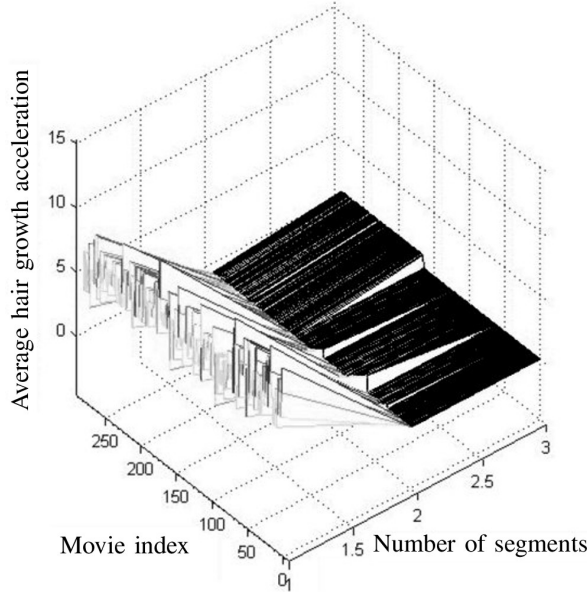


Fig. 3: Projection of the examined movies on two features, “number of segments” and “average hair growth acceleration”. Points are colored based on class ID; black color is Class 0 and shades of gray are Class 1.

4. Discussion and Related Research

Among higher plants, the model organism *Arabidopsis Thaliana* has been studied in detail. As its genome is sequenced and better understood than other similar plants, there are many opportunities for investigation of the genotype-phenotype mapping. The development of the Arabidopsis root system is a centerpiece in plant biology. Due to the wide availability of a large amount of literature on root development, the Arabidopsis root system continues to serve as an excellent model organ to investigate systems biology of higher plants. The functional landscape of gene-protein network dynamics is believed to be responsible for the regulation of growth and development of roots. Multi-scale mathematical modeling of root growth provides a critical element for a systematic study of the mechanisms of regulatory transcription networks that operate on different scales, specific interactions of numerous proteins and tightly intertwined protein interaction networks. Gene expression

studies need to be performed at temporal and spatial resolutions (minutes and micrometers) that are relevant to the dynamics of gene and phenotype crosstalk. Historically, tropic growth responses have been at the center of such activities. Tropic responses allow plants to redirect their growth in response to their surrounding environment. The temporal dynamics of root growth is relatively accurately measured by following the displacement of features at extremities, such as the tips of roots and root hairs. It is necessary to obtain velocity profiles of neighboring elements as they are moved by expansion [19], [36]. Velocity profiles can be obtained by imaging a growing organ over time which provides measures of the position of the externally applied marks, thus computing velocity as a function of position [23].

The mathematical analysis of image sequences goes back to numerous early investigators, e.g. [20], [22], and algorithmic methods substitute for laborious and often subjective manual measurements ([25], [7], [34], [42]). Several improvements were made by developing algorithms that measure the spatial growth profiles using image-processing techniques that could also utilize cell borders, intercellular air spaces or other physiological structures that are followed throughout a sequence of images (see [24], [29], [35], [10]). Concepts originally developed by [27], [28] and [45], [46] and improved by [10] establish quantitative relationships for curvature production and curvature angle distribution. Nevertheless, there is a continuing need to develop better algorithms that work more accurately in automated high throughput imaging systems such as our system outlined above.

5. Conclusion

High throughput plant imaging systems require appropriate software applications for accurate automated image analysis. We have developed a prototype hardware-software product that operates as a “*Portable Modular System for Automated Image Acquisition and Analysis*” in the lab and some field applications. This article provides evidence for feasibility of using our system towards fully automated high throughput phenotyping in challenging functional genomics and systems biology applications. The methods described above also point to the many opportunities that machine learning could offer plant functional biology, such as distinguishing mutant from wild type seedling plants in gravitropism experiments. As we observed, these applications could be adapted for other experimental protocols in plant biology that attempt to quantify subtle phenotypic traits in order to decipher the functions of genes-proteins. The general problem of quantifying *all* phenotypic traits (for example, in tropism) for the development and growth of the plant root system remains a formidable challenge that is certain to inspire new insights in machine learning and analysis of massive biological imaging data.

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